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Excystation of *Isospora suis* Biester, 1934 of Swine

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Abstract. The in vitro excystation of sporozoites of *Isospora suis* Biester 1934 is described. Sporocysts of *I. suis* lack a Stieda body. Upon incubation in 0.75% sodium taurocholate or in 0.25% trypsin + 0.75% sodium taurocholate excystation solutions, sporozoites were released by separation of the sporocyst wall into four plates. Occasionally, the sporocyst wall did not separate completely but opened partially and released the sporozoite. At the time of excystation, sporozoites were short and broad but became elongated after 5 to 10 min in the excystation fluids. Elongate sporozoites measuring 11.7 × 3.8 μm, had a pointed anterior end and a nucleus located in the posterior half of the cell. Living sporozoites exhibited gliding movements, side-to- side flexion, and probed with their anterior ends. Incubation in 5.25% sodium hypochlorite removed the oocyst walls from most oocysts. Sporozoites did not excyst from sporocysts that were released during treatment with sodium hypochlorite.

Introduction

Some species of *Isospora* are similar to *Eimeria* spp. in that excystation involves dissolution of a Stieda body and escape of sporozoites through the small opening left in the end of the sporocyst wall (Duszynski and Brunson 1972; Speer and Duszynski 1975). In contrast, several species of *Isospora* without a Stieda body in one end of their sporocysts have been shown to excyst by collapse of sporocyst wall plates (Speer et al. 1973; Duszynski and File 1974; Duszynski and Speer 1976).

Endogenous development (Lindsay et al. 1980) and sporogony (Lindsay et al. in press) of *Isospora suis* Biester 1934 has been described. The present paper provides additional information about the life cycle of this economically important pathogen of neonatal pigs (Stuart et al. 1980; Eustis and Nelson 1981).

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Materials and Methods

Oocysts of *I. suis* were obtained from experimentally infected pigs, sporulated in 2.5% potassium dichromate solution (Lindsay et al. 1980) and stored at 4° C for 60 to 70 days before being used in the present studies. Oocysts were cleaned and concentrated using Sheather's sugar solution (Lindsay et al. 1980), washed by centrifugation, and suspended in Ringer's "warm-blooded" saline (RWS).

Groups of oocysts were processed by four diferent methods: (a) treatment with 5.25% sodium hypochlorite (NaOCl) for 15 min in an ice bath; (b) treatment with 2.63% NaOCl for 15 min in an ice bath; (c) grinding with a teflon coated tissue grinder to release the sporocysts; and (d) resuspending untreated oocysts in RWS and leaving them in this solution. Oocysts in groups 1 and 2 were washed four times and resuspended in RWS before addition of the test excystation solutions.

Untreated oocysts, oocysts treated with NaOCl, and sporocysts released by grinding were each incubated at 37° C in four different test solutions. The solutions were: (a) 75% sodium taurocholate (bile salt) in RWS, (b) 0.25% trypsin + 0.75% sodium taurocholate (trypsin-bile salt) in RWS, (c) 0.25% trypsin in RWS; and (d) RWS alone. Samples from each oocysts or sporocyst preparation in each test solution were examined by Nomarski interference contrast microscopy at 5-min intervals until excystation was complete. If sporozoites were not released within 2 h, the solution was considered incapable of stimulating excystation. To judge the efficacy of excystation of untreated oocysts and of oocysts treated with NaOCl, 200 of these stages were examined after 2 h incubation at 37° C, and the percentage of oocysts which had released sporozoites was determined.

Fifty living sporozoites were measured at the end of 2-h incubation in the trypsin-bile salt solution. Measurements were also made of methanol-fixed and Giemsa-stained sporozoites. All measurements were made with a calibrated ocular micrometer. In all instances the means are followed by ranges in parentheses.

To test the effects of age of oocyst on excystation, oocysts stored in 2.5% potassium dichromate solution for 30 and 260 days were also processed like group c above, and incubated in trypsin-bile salt solution.

Results

Some untreated oocysts had walls which appeared wrinkled or cracked when examined before addition of excystation solutions (Fig. 1). Sporocysts inside these oocysts were morphologically normal. Excystation solutions did not cause any visible change in the oocyst walls.

Treatment with 5.25% or 2.63% NaOCl for 15 min removed the oocysts walls from most oocysts. However, some remained completely intact and some had only intact inner oocyst walls surrounding the sporocysts (Fig. 2). Most sporocysts released from oocysts treated with 5.25% or 2.63% NaOCl appeared morphologically normal but a few had areas in the sporocyst wall that were collapsed. All sporocysts released from NaOCl-treated oocysts failed to excyst when incubated in any of the excystation solutions. Also sporozoites freed mechanically from thus treated sporocysts were non-motile and appeared dead. However, sporocysts not released from oocysts treated with 5.25% or 2.63% NaOCl behaved as those in untreated oocysts when incubated in excystation solutions.

Excystation occurred in untreated, as well as in 5.25% and 2.63% NaOCl-treated oocysts, with one or both walls intact, after incubation for

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10 to 20 min in bile salt or trypsin-bile salt solution. After 2-h incubation in the former solution, 35% of untreated oocysts, 11% of oocysts treated with 5.25% NaOCl, and 28% of those treated with 2.63% NaOCl released sporozoites from their sporocysts. After 2-h incubation in trypsin-bile salt solution, 26% of untreated oocysts, 15% of those treated with 5.25% NaOCl, and 32% of those treated with 2.63% NaOCl released sporozoites from their sporocysts. Sporozoites were released from mechanically freed sporocysts after 5–10 min incubation in bile salt or trypsin-bile salt solution. After 2-h incubation in either of these solutions, approximately 95% of the mechanically freed sporocysts released their sporozoites. No excystation occurred in untreated oocysts, intact oocysts treated with 5.25% of 2.63% NaOCl, or mechanically freed sporocysts after 2 h in RWS or in the 0.25% trypsin solution.

No differences were seen in the method of releasing sporozoites from sporocysts which were 30, 60 to 70, or 260 days-old. Approximately 95% of the 30- or 60-to 70-day-old sporocysts and 90% of those 260-days-old released sporozoites after incubation in trypsin-bile salt solution.

Before excystation, sporozoites swelled slightly and exhibited intermittent tumbling movements inside the sporocysts. Indentations in the sporocyst wall could be seen shortly after these movements began (Fig. 3). Most sporocyst walls then separated quickly into four plates releasing the sporozoites (Figs. 4, 5). Less frequently, the sporocyst wall opened only partially and sporozoites emerged through the opening (Figs. 6, 7). Simultaneous excystation of sporozoites from both sporocysts within an oocyst was not observed. Occasionally an oocyst was seen with one sporocyst from which sporozoites excysted by complete collapse of plates and the other which released sporozoites by partial separation of the plates (Fig. 8). Sporozoites released from sporocysts that were surrounded by an oocyst wall were observed moving freely inside the oocyst. Eventually, they escaped through a break in the oocyst wall. Sporozoites apparently could not escape from oocysts with intact walls (Fig. 9).

Freshly excysted sporozoites were short and broad; they were weakly motile (Fig. 10). Five to 10 min after excystation, sporozoites became elongate and highly motile. Elongate sporozoites (Fig. 11) had a pointed anterior end and a rounded posterior end, with a nucleus located within the posterior half. Small refractile granules were scattered throughout these stages. Movements of such sporozoites consisted of rapid forward gliding, side-to-side flexion, and a probing action with the anterior end. No refractile bodies were seen in any of these stages. Fifty elongated sporozoites measured 11.7 (10–13) × 3.8 (3–5) μm.

Giemsa-stained sporozoites (Fig. 12) resembled living sporozoites with regard to the position of the nucleus and the presence of scattered granules in the cytoplasm. The pointed anterior end seen in living sporozoites was not evident in Giemsa-stained organisms, in which no refractile bodies were noted. Fifty Giemsa-stained sporozoites measured 9.9 (8–12) × 2.8 (2–5) μm.

Discussion

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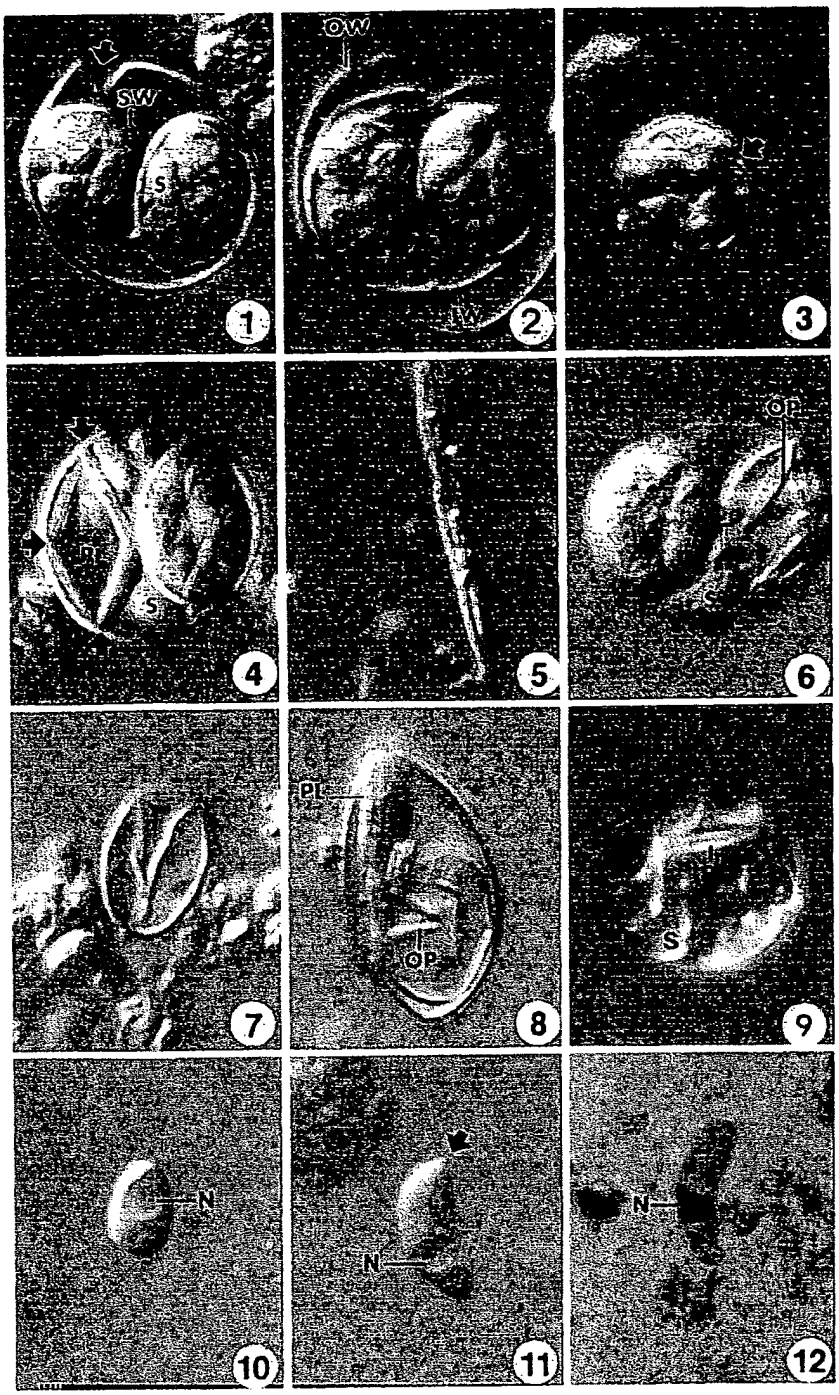

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Fig. 11. Elongate sporozoite w
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Fig. 12. Giemsa-stained sporoz
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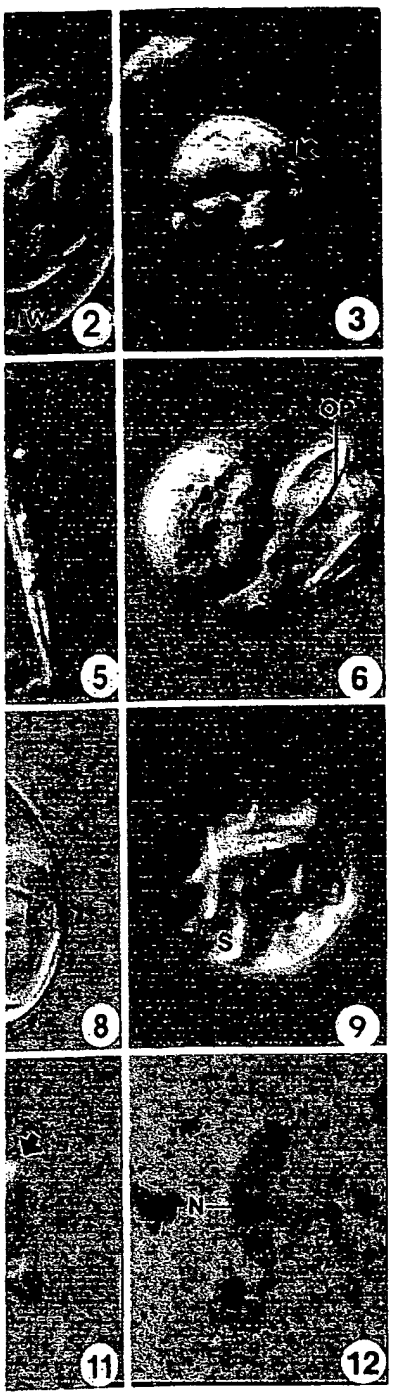
anterior end

Discussion

The abnormalities (wrinkles and/or cracks) observed in some of the oocyst walls of untreated *I. suis* oocysts before addition of excystation solutions were probably due to storage and concentration techniques. These abnormalities were not observed in oocyst walls of freshly sporulated oocysts (Lindsay et al. in press). In the present study, the morphologic abnormalities in the oocyst wall had no apparent physiologic effect on the excystation process, since approximately 95% of the sporocysts freed mechanically from oocysts stored for 30 or 60–70 days and approximately 90% from those stored for 260 days released sporozoites when incubated in trypsin-bile salt solution. In addition, oocysts stored for 260 days in 2.5% potassium dichromate produced clinical coccidiosis in susceptible piglets which was indistinguishable from that produced by inoculating the same number of oocysts



Figs. 1–12. Nomarski interference contrast (Figs. 1–11) and bright field (Fig. 12) photomicrographs of oocysts, sporocysts, and sporozoites of *Isospora suis* before (Figs. 1, 2), during (Figs. 3–10), and after (Figs. 11, 12) incubation in excystation solution. All Figures, × 1,600

Fig. 1. Untreated oocyst showing a break (arrow) in the oocyst wall. *S* sporozoite; *SW* sporocyst wall.

Fig. 2. Oocyst pretreated with 5.25% NaOCl. Note the intact inner wall *IW* and portion of the removed outer wall *OW*

Fig. 3. Mechanically freed sporocyst showing an indentation (*arrow*) in the sporocyst wall

Fig. 4. Untreated oocyst in which one sporocyst has separated into four plates. Arrows point to sites where plates *PL* are still joined. Several released sporozoites *S* can be seen within the oocyst

Fig. 5. Sporocyst wall plates remaining after release of sporozoites from a mechanically freed sporocyst

Fig. 6. Oocyst, pretreated with 5.25% NaOCl, containing an intact sporocyst and another one which has partially opened and released its sporozoites *S*. Opening in sporocyst wall *OP*

Fig. 7. Mechanically freed sporocyst which has opened partially and released its sporozoites

Fig. 8. Untreated oocyst containing one sporocyst with collapsed wall plates *PL* and another with partly opened wall plates *OP*.

Fig. 9. Untreated oocyst 2 h after incubation. Both sporocysts have collapsed wall plates *PL*, but the released sporozoites *S* have not left the oocyst

Fig. 10. Freshly excysted, short, broad sporozoite with a prominent nucleus *N*

Fig. 11. Elongate sporozoite with a pointed anterior end (arrow) and a nucleus *N* in the posterior half of the cell

Fig. 12. Giemsa-stained sporozoite showing granules scattered within the cytoplasm and a nucleus *N* located just posterior to the middle of the cell. Note the absence of a pointed anterior end

stored for 30 or 60–70 days. Similarly, storage in potassium dichromate of *I. canis* oocysts for 56 days (Speer et al. 1973) and *I. arctopitheci* oocysts for 100 days (Duszynski and Speer 1976) had no adverse effects on sporocyst structure or on the excystation process.

Complete removal of the oocyst walls by 5.25% or 2.63% NaOCl-treatment indicates that oocysts of *I. suis* are more sensitive to NaOCl than these forms of many species of *Eimeria* and *Isospora* in which similar treatment removes only the outer wall (Nyberg and Knapp 1970; Roberts et al. 1970; Speer et al. 1973; Dubey 1975, 1979; Dubey and Fayer 1976). Pretreatment of oocysts of *I. felis* (Fayer and Thompson 1974), *I. ohioensis* (Dubey 1975), *I. rivolta* (Dubey 1979), and *I. bigemina* (Dubey and Fayer 1976) with NaOCl did not adversely affect their infectivity for, and development in susceptible hosts and cell cultures. In the present study, direct exposure of *I. suis* sporocysts to 5.25% or 2.63% NaOCl solutions apparently killed the sporozoites, as they were nonmotile and failed to excyst when incubated in the excystation solutions. Also, when sporocysts previously exposed to 5.25% or 2.63% NaOCl were washed, suspended in RWS, and mechanically ruptured, the sporozoites released were nonmotile and appeared dead. Some protection from the adverse effects of NaOCl was provided for sporocysts within oocysts which retained the inner wall, since approximately 11% contained sporocysts capable of releasing viable sporozoites when placed in excysting solutions. Storage of sporocysts of *Sarcocystis cruzi* in a 1% NaOCl solution greatly reduced the percent of excystation in comparison to that recorded for sporocysts stored in distilled water or in a balanced salt solution (Leek and Fayer 1979). Results obtained with *I. suis* (present study) and those derived from studies of *S. cruzi* (Leek and Fayer, 1979) indicate that sporocysts of both these species are more sensitive to NaOCl than are the oocysts of most species of *Eimeria*.

The excystation process of *I. suis* is similar to that described previously for *I. canis* (Speer et al. 1973), *I. endocallimici* (Duszynski and File 1974), *I. arctopitheci* and *I. bigemina* (Duszynski and Speer 1976), as well as for *Toxoplasma gondii* (Christie et al. 1978) and *Sarcocystis* spp. (Box et al. 1980), all of which lack a Stieda body and excyst by separation of sporocyst wall plates. Simultaneous release of sporozoites from both sporocysts within an oocyst was seen in *I. canis* (Speer et al. 1973) and *T. gondii* (Christie et al. 1978), but not in *I. endocallimici* (Duszynski and File 1974) or in *I. suis* (present study). Intact oocysts of *I. endocallimici* (Duszynski and File 1974) and *I. arctopitheci* (Duszynski and Speer 1976) did not excyst when exposed to trypsin-bile salt solutions. Untreated oocysts of *I. canis* excysted after prolonged incubation in excystation solution, whereas NaOCl-treated oocysts excysted after a much briefer incubation (Speer et al. 1973). In the present study, the time of initiation of excystation was the same for untreated and 5.25% and 2.63% NaOCl-treated oocysts. The wrinkles and cracks observed in the walls of some untreated oocyst (Cf. Fig. 1) probably allowed entrance of excysting solution which then stimulated excystation. No excystation occurred within oocysts with normal-appearing walls and no visible alteration in the oocyst wall occurred when the oocysts were incubated

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in excystation solutions. In the present study, bile salt solution was shown to be the only stimulus necessary for excystation of *I. suis*. Similar observations were reported for *I. canis* (Speer et al. 1973) and *T. gondii* (Christie et al. 1978). Trypsin solution alone was not sufficient to cause excystation of *I. suis* (present study) or of *I. canis* in a previous study (Speer et al. 1973). Roberts et al. (1970) reported that incubation in trypsin solution was the only treatment necessary for excystation of mechanically freed sporocysts of *Eimeria callospermophili* and *E. larimerensis* and that a bile salt solution alone caused movement of the sporozoites within sporocysts but not excystation. In sporocysts which lack a Stieda body, excystation is accomplished by separation of sporocyst wall plates. Since treatment with a proteolytic enzyme is not necessary for excystation of *I. suis*, it is possible that movement of the sporozoites inside sporocyst exerts pressure on the sporocyst wall plates, causing them to separate and to release the sporozoites.

Sporozoites of *I. suis* resemble those of *I. arctopitheci* (Duszynski and Speer 1976) in that both are short and broad upon release from the sporocysts, but become elongate after a brief incubation period. The short forms of *I. arctopitheci* sporozoites contain a relatively large dense body in the cytoplasm which disappears after elongation. No such body was seen in the short or elongate forms of *I. suis* sporozoites (present study).

Of the coccidia of warm blooded animals with known life cycles, those whose sporocysts contain a Stieda body are homoxenous whereas those whose sporocysts are composed of plates have an intermediate host (Box et al. 1980). Since excystation structures appear to correlate with life cycles, Box et al. (1980) proposed that *Isospora* spp. with Stieda bodies be placed in the Eimeridae and that *Isospora* spp. without Stieda bodies be placed in the Sarcocystidae. If this proposal is accepted, *I. suis* would be transferred to the genus *Cystoisospora*. Frenkel's (1977) original description of the genus *Cystoisospora* was based on the presence of monozoic cysts in intermediate hosts. Since *I. suis* has not been shown to have such cysts, reclassification of this parasite would be premature.

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The Chetotaxy of of *Opisthioglyphe* (Trematoda, Plagi

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Abstract. The distribu studied for the cercar emerge from the fresh according to the meth papillae were demons The results were identi *ius corneus* from a p fected, laboratory-rea distributed symmetrica of the trematode with by the chetotaxy of th of the cercaria from th system of the cercaria teristics with other cer like the chetotaxy, emp

Introduction

The sensory receptors (teg todes lie on the body sur system. Their distributio of a trematode species. E is regarded as unchanged and taxonomical value is a cinskaja and Dobrovol'ski cal investigation of the t the beginning, and inform

Opisthioglyphe locellus corneus L. as first host a insects as second interme